Was He Murdered or Was He Not?-Part II

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WAS HE MURDERED OR WAS HE NOT?—PART II: MULTI-ELEMENTAL ANALYSES OF HAIR AND BONE SAMPLES FROM TYCHO BRAHE AND HISTOPATHOLOGY OF HIS BONES*

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Hair and bone samples procured from the remains of Tycho Brahe were analysed by several analytical techniques. In segmented hair samples, concentrations of Fe, As, Ag and Au at the tips exceeded values for the contemporary population; however, they decreased towards the hair bulbs, similarly to Hg, indicating that recent exposure that was discontinued ~2 months prior to Brahe's death. Several other elements did not follow this pattern. Analyses of bones revealed signs of long-term exposure to Au, while many other elements were within expected ranges. Histopathological examination of bone sections yielded no signs of severe bone metabolic disorders.

KEYWORDS: TYCHO BRAHE, HAIR, BONES, TRACE ELEMENTAL ANALYSIS, TIME TRENDS, BONE HISTOPATHOLOGY

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INTRODUCTION

In 2010, a bi-national research team unearthed the remains of renaissance astronomer Tycho Brahe from his resting place in the Church of Our Lady before Týn (Týn Church) in Prague. A number of samples of hair, teeth, bone and textile were procured for analysis before the remains were re-buried. The first set of analyses excluded that Brahe received lethal or even moderate doses of Hg, both shortly before and several years prior to his death (Rasmussen *et al.* 2013c). The suspicion of Hg poisoning has been raised repeatedly, starting shortly after his death. Brahe, however, took an active interest in alchemy (e.g., Dreyer 1963), like several other outstanding scientists of the 17th century—for example, Sir Isaac Newton (Keynes 1995) and Robert Boyle (Boyle 1661)—an activity that could have led to excessive intake of Hg. As it turned out, this was not the case for Brahe. It is not known whether Brahe actually participated in alchemical experiments himself or whether he just had an interest on a more theoretical level. If Brahe did take part in alchemical experimentation, it is possible that even though he was not exposed to Hg, he could have been exposed to various other chemical substances used in alchemy. Another possible route of exposure was through preparing elixirs, for which Brahe was famous (Janovský 2010), or their self-administration.

In the present work, we provide additional observations and chemical analyses of samples of his hair, beard, eyebrows and bones. Besides Hg, the analytes include Mg, Al, Cr, Mn, Fe, Co, Cu, Zn, As, Sr, Ag, Sb, Ba, Pb and Au. The analyses were performed using multi-elemental instrumental neutron activation analysis (INAA), radiochemical (destructive) neutron activation analysis (RNAA), inductively coupled plasma – mass spectrometry (ICP–MS), cold vapour atomic absorption spectrometry (CV–AAS), and micro-proton-induced X-ray emission (μ-PIXE). INAA was performed prior to Hg determination by RNAA (Rasmussen *et al.* 2013c). We also provide results of histopathological examinations of bones, which add to the description of the health and physiological status of Tycho Brahe at the time of his death. The analyses conducted help to elucidate the life and death of Tycho Brahe. His death has attracted particular attention as being suspicious, due to a short illness, right from the start and continuing until recent years. But even besides the implications for his death, the investigations of Brahe's remains are interesting because of his lifelong activities in natural sciences including alchemy—the dawn of modern chemistry.

EXPERIMENTAL

Table 1 lists the samples assayed. Hair samples were analysed at the Nuclear Physics Institute of the Czech Academy of Sciences (NPI) and bone samples were analysed both at NPI and at the University of Southern Denmark (SDU), whereas bone histopathology was carried out at the Charles University in Prague (CUP).

Analyses of hair samples

Hair samples were assayed by INAA and RNAA at NPI, within the CANAM infrastructure (MŠMT project LM 2011019). The hair specimen TB77 was collected when the tomb was first opened in 1901 (Matiegka 1901) and then deposited in the collections of the City of Prague Museum. Other samples of scalp hair, beard hair and eyebrow hair were collected during the second opening of the grave in 2010 (TB38, TB39 and TB40). Some of these samples were glued together by adhering soft tissue (e.g., mummified skin, dried blood). Therefore, the samples were

Table 1 *Samples of remains of Tycho Brahe analysed by INAA, RNAA, CV–AAS and ICP–MS. All samples, except for TB77, were obtained during the tomb opening in November 2010. TB77 was sampled during the previous opening of the grave in 1901 and obtained from the collections of the City of Prague Museum*

Sample code	*Description*	*Mass (mg)**	*Mass (mg)†*
TB38–1	Hair, 0–5 mm from the bulb‡	0.280	
TB38–2	Hair, 5–10 mm from the bulb§	0.227	
TB38–3	Hair, 10–15 mm from the bulb¶	0.244	
TB38–4	Hair, 15–20 mm from the bulb‖	0.225	
TB38	Bulk hair	4.291	
TB3 8 t	Tissue adherent to collected hair TB38	0.718	
TB39–1	Beard hair, 0–5 mm from the bulb‡	0.629	
TB39–2	Beard hair, 5–10 mm from the bulb§	0.584	
TB39–3	Beard hair, 10–15 mm from the bulb¶	0.478	
TB39	Bulk beard hair	2.695	
TB40	Bulk eyebrow	0.237	
TB77–1	Hair, 0–5 mm from the bulb‡	0.295	
TB77–2	Hair, 5–10 mm from the bulb§	0.306	
TB77–3	Hair, 10–15 mm from the bulb¶	0.285	
TB77–4	Hair, 15–20 mm from the bulb‖	0.253	
TB77	Bulk hair	0.825	
TB55	Os ilium (sin), trabecular		20.4
TB56A	Os ilium (sin), cortical part	39.61	
TB56B	Os ilium (sin), trabecular part	167.02	
TB57a	Femur (sin)		20.9
TB58	Femur (sin), sample from subtrochanteric area	163.80	

*Analysed at NPI.

†Analysed at SDU.

‡Denoted as '0–14 days before death' in Figures 1 and 2.

§Denoted as '15–29 days before death' in Figures 1 and 2.

¶Denoted as '30–44 days before death' in Figures 1 and 2.

‖Denoted as '45–59 days before death' in Figures 1 and 2.

cleaned as described previously (Rasmussen *et al.* 2013c). First, the adherent tissue was removed mechanically. The tissue removed from sample TB38, which was available in a sufficient amount, has been labelled TB38t and retained for analysis. After mechanical cleaning, the hair samples, typically 20 mm long for TB38 and TB77, and 10–15 mm long for TB39, with well-identified bulbs, were cut into 5-mm segments and washed using the procedure described in Ryabukhin (1978). Since the eyebrow hairs were relatively short, 5–8 mm long, they were not cut into shorter sections, but analysed as a bulk sample. A portion of the hair samples TB38, TB39 and TB77 with no clearly identified bulbs (broken hairs etc.) were also analysed as bulk samples. The segmented hair samples and bulk sample TB40 were sealed in pre-cleaned quartz ampoules (Suprasil® AN Heraeus), irradiated in the LVR-15 reactor at Řež at a thermal neutron fluence rate of $3 \times 10^{13}\,\mathrm{cm^{-2}\,s^{-1}}$ for 20 h, decomposed in 4 mL of dilute nitric acid (1 + 1) and adjusted to a total volume of 10 mL using deionized water, as described in detail in Rasmussen *et al.* (2013c). The quantification was performed using k_0 standardization (k_0-INAA) prior to mercury separation for destructive determination by RNAA according to Kučera and Soukal (1993). The neutron flux monitors for k_0-INAA were irradiated for 2 h shortly before and shortly after the 20-h irradiation of the samples and the monitor activities were counted according to the

standard protocol as described in Kubešová and Kučera (2010). The irradiated samples were counted using high-efficiency, high-resolution high-purity germanium (HPGe) detectors after decay times of 3–4 days and about 1 month to determine the elements forming medium- and long-lived radionuclides, respectively. INAA with relative standardization was used for assay of bulk hair and beard samples, as described by Kučera and Soukal (1988). The samples were packed for irradiation in pre-cleaned polyethylene (PE) capsules. First, they were irradiated for 1 min in the above conditions to determine elements forming short-lived radionuclides. The induced activity was measured with a HPGe detector for 10 min after 10 min of decay. After 5 days, the samples were re-irradiated for 2 h. Gamma-ray spectrometry counting of the induced activities was carried out after the same decay times as given above. For quality control purposes, about 150-mg aliquots of NIST SRM 1515 Apple Leaves were analysed simultaneously with the samples.

Lead was determined in one hair from samples TB77 and TB38 by μ-PIXE, using a Tandetron 4130 MC accelerator with a 2.6 MeV proton beam, focused to a diameter of 1.5 μm. Multiple scans were performed over 500 μm sections of hair at several distances from the hair bulb, using a 0.1 nA beam current for 1–3 h. The quantification was carried out using the GUPIX computer code (Maxwell *et al.* 1989) from Pb L-lines. The detector was calibrated using a set of thick targets and the proton dose was determined from the RBS spectra using the Q factor method (Grime 1996). The mean hair thickness of 45 μm was considered in order to correctly evaluate attenuation and proton energy loss corrections. Finally, the results were normalized against the S content, with a reference value of 6.2 mass per cent.

Analyses of the bone samples

At SDU, the bone samples were analysed by CV–AAS to determine Hg (Rasmussen *et al.* 2013c) and by ICP–MS to determine several other elements (Skytte and Rasmussen 2013). The surfaces of the bone samples were removed mechanically using a DremelMultiPro electric drill model 395 with an adjustable drilling velocity and equipped with a 2-mm diameter drill. Prior to sampling, the drill and all other stainless steel utensils that were likely to get in contact with the sample were rinsed in ethanol and heated in an ethanol flame in order to evaporate any Hg possibly present as contamination. On the cortical bone, a surficial layer of ~1 mm of the bone was removed and discarded. Then, the drilling of the sample material was repeated to collect a sample portion for analysis. On the sample of trabecular bone, the same procedure was applied, except that here no surficial layer was removed.

A further step in the decontamination procedure of bone samples has been added compared with the procedure previously described by Rasmussen *et al.* (2013c). It is often necessary to mechanically remove dark particles from the drilled bone sample (Rasmussen *et al.* 2015) under the microscope, using rinsed steel tweezers. The dark particles, mainly consisting of silicates, decayed soft tissue or diagenetic deposits, are often present in samples of trabecular tissue, because it is impossible to decontaminate the surface of a trabecular bone sample during the drilling operation. Rasmussen *et al.* (2015) concluded that a Ca concentration of 22.1 wt% can be used as a threshold value; that is, samples showing Ca concentrations below this threshold value must be considered to be contaminated and must be deleted from the data set. An aliquot of the sample of trabecular tissue TB55/KLR-8244, reported in Rasmussen *et al.* (2013c), was analysed by ICP–MS and the Ca concentration was found to be 20.4 wt%, which is below the threshold value of 22.1 wt%. Accordingly, a new sample was drilled from TB55 and the decontaminated sample

was analysed by both CV–AAS and ICP–MS. The Ca concentration was now found to be 24.0 wt% (cf., Table 3 below).

Each sample portion of ~40 mg was dissolved in a mixture of 4 mL of HNO_3, 2 mL of H_2O_2 and 0.5 mL of HCl, all of ICP–MS grade, in sealed polystyrene containers on a shaking table for 24 h at room temperature (~20 °C). The samples were then diluted to 10 mL with Milli-Q water and filtered through 0.45 μm disposable filters. After filtering, half of the solution was used for ICP–MS, and the other half for CV–AAS for Hg. The solutions were stored at +4 °C until the analyses were performed. The analyses were carried out using a Bruker ICP–MS 820 spectrometer equipped with a frequency-matching RF generator and a skimmer gas Reaction System (CRI), operating with helium or hydrogen in order to avoid interference with polyatomic species. The CRI Reaction System was activated for ^{52}Cr, ^{60}Ni and ^{75}As using He as skimmer gas, and for ^{56}Fe using H_2. In all analyses, ^{45}Sc, ^{89}Y and ^{159}Tb were used as internal standards. The rest of the elements were analysed without skimmer gas. Interfering masses have been corrected for in all relevant cases, using an algorithm in the software. Multi-element calibration standards were prepared in 1% HNO_3 at six different concentrations (0, 1, 10, 20, 100 and 200 $\mu g\,L^{-1}$), but for each element only three of these standards were selected to fit the appropriate concentration range.

At NPI, the surfaces of bone samples analysed were removed by scraping with a stainless steel scalpel. The bones, with a mass of ~500 mg, were pulverized using agate ball-mill Pulverisette 5 (Fritsch) for 10 min and later analysed using the same procedure that was used for the bulk hair samples. The additional sample cleaning under a microscope was not performed at NPI, but the quantity of the processed bone samples was larger than at SDU and thus a lesser effect of the surface contamination should be expected.

Histological examination of bone

A sample with both cortices was removed from the anterior part of the ilium, composed of the medial and lateral cortical bone, including cancellous bone. An anterior iliac crest sample was used, because this site is considered to be optimal for bone histomorphometric examination. The specimen was fixed in 70% ethanol for 48 h. Then, it was dehydrated in absolute alcohol and embedded in methyl methacrylate based resin; subsequently, thin sections were cut from the sample using a Leica SM 2500 microtome and stained by several techniques. Five micron sections were stained with hematoxylin–eosine, Masson's trichrome, von Kossa's impregnation method, toluidine blue, histochemical staining reactions for Al (aluminon technique) and Fe (Perls' method). Qualitative and quantitative analysis of sections using light and polarized light was performed.

The basic quantitative histomorphometric analysis of trabecular bone was carried out using the following calculations: trabecular bone volume (TV/BV) = bone volume (BV)/tissue volume (TV) × 100 (%), corresponding to a percentage of the total bone tissue volume; osteoid relative surface (OS/BS) = trabecular surface covered with osteoid (OS)/all trabecular surface (BS) × 100%, corresponding to a percentage of the trabecular bone surface covered with osteoid. The trabecular diameter was the shortest distance from one surface to the other. The median value of at least 100 measurements was used to define the trabecular diameter.

Measurements were made by semi-automated image analysis using a Quick photo camera from Olympus and a Laboratory Imaging Lucia image analyser. All morphometric measurements were done in the spongy bone of the iliac crest. The field for morphological evaluation was 30 mm^2.

RESULTS AND DISCUSSION

Hair samples.

INAA results for hair samples and quality control results are shown in Table 2. Our values for NIST SRM 1515 compare well with the NIST values, thus proving the accuracy of our results. The accuracy of Hg determination by RNAA has been reported elsewhere (Rasmussen *et al.* 2013c).

Hair provides a record of exposure to trace elements in the range of months (Rasmussen *et al.* 2013c and references therein). Considering the most frequently cited hair growth of 10 mm per month and the length of most of the analysed hair filaments, the segments analysed in the present study cover the exposure over approximately 2 months prior to Brahe's death. Concentrations as a function of time for the elements Cr, Fe, Co, Zn, As and Br in the hair segments are shown in Figure 1, while those of Ag, Sb, Au, Hg and Pb are given in Figure 2. The literature mean values (median or arithmetic mean—whatever is available) for modern man and ranges for the contemporary, occupationally non-exposed population are also shown in Figures 1 and 2.

Some elements occur in concentrations similar to those found in modern humans; that is, Cr, Zn, Br, Sb, Hg and Pb. However, other elements were found in elevated concentrations, namely Fe, Co, As, Ag and Au. It therefore seems that Tycho Brahe was excessively exposed to the latter group of elements in the last 2 months of his life.

Two types of time developments of the concentrations in the hair of Tycho Brahe can be identified: (i) those with little or erratic variation with time—that is, Cr, Co, Zn, Br, Sb and Pb; and (ii) elements that decrease in concentration during the last 2 months of the life of Tycho Brahe—Fe, As, Ag and Au, where the initial values exceed those of the present population, and Hg, which lies within the range for the contemporary population but with a concentration that still decreases towards his death.

The concentrations in the bulk hair samples given in Table 2 are, in the majority of cases, within the ranges of values determined in the segmented hair samples. The results for the three hair samples analysed were mutually comparable, with some exceptions. The beard hair (TB39) exhibited an order of magnitude higher Mn and Cu content than the other samples. The high Cu content in TB39 may be due to corrosion of Brahe's nose prosthesis, which was probably made of brass (Rasmussen *et al.* 2013c), resulting in enrichment of Cu on the brass surface (El-Mahdy *et al.* 2013). The hair sample from the museum collection (TB77) had an exceptionally high Br content. Many specimens in museum collections have been conserved using a range of chemical compounds, some of which contain As, Br or Hg. Hawks (2001) reported the use of methyl bromide or ethylene dibromide, which are used for pest or mould control. It therefore appears likely that TB77 has been treated with a Br-containing compound.

Elemental concentrations in the skin tissue (TB38t) are higher for several elements compared to the hair samples, namely for Fe, Co, Cu and Br. This demonstrates that proper mechanical and wet cleaning of the hair samples prior to analysis was essential for removing contamination and for obtaining reliable data for the endogenous element contents. The concentrations of Ag, Sb, Au and Hg in TB38t were about the same as or lower than those found in most of the hair samples, and thus could not cause significant contamination in any case.

In comparison to elemental concentrations for modern populations (cf., column 6 in Table 2), the hair samples from Brahe, TB38 and TB77, were deficient in Zn, which might indicate a lower intake of this element compared to modern man. On the other hand, Brahe's hair contained elevated levels of Fe, Co, Cu, As, Ag and Au. The elevated concentrations and the already

Table 2 *Element contents in bulk hair samples (scalp hair TB38 and TB77, beard hair TB39 and eyebrow hair TB40) and tissue adherent to sample TB38, in $\mu g\,g^{-1}$, determined by INAA, literature values for human hair and quality control results for NIST SRM 1515 Apple Leaves*

Element	*TB38* $x_i \pm u_i$[†]	*TB77* $x_i \pm u_i$[†]	*TB39* $x_i \pm u_i$[†]	*TB40* $x_i \pm u_i$[†]	*Literature value* *Median (range)*[‡]	*TB38t* $x_i \pm u_i$[†]	*NIST SRM 1515* $x \pm s$ ($n = 3$)	*NIST value**
Cr	1.8 ± 0.8	<1.5	2.3 ± 0.8	6.6 ± 1.0	0.46 (0.06–4.10)[§]	2.7 ± 0.6	0.25 ± 0.02	0.30
Mn	1.60 ± 0.11	1.1 ± 0.2	17.2 ± 0.6	NA	1.2 (0.2–4.4)[§]	NA	55.08 ± 0.13	54 ± 3
Fe	350 ± 50	620 ± 150	790 ± 60	<200	33 (13–177)[§]	6200 ± 200	76 ± 4	83 ± 5
Co	2.82 ± 0.14	1.5 ± 0.3	1.16 ± 0.11	0.51 ± 0.07	0.077 (0.0004–0.50)[§]	33.8 ± 1.3	0.092 ± 0.002	0.09
Cu	102 ± 8	54 ± 7	1940 ± 60	1920 ± 90	16 (6.8–39)[§]	880 ± 80	<6	5.64 ± 0.24
Zn	31 ± 3	63 ± 8	157 ± 5	534 ± 20	175 (124–320)[§]	247 ± 9	11.5 ± 0.4	12.5 ± 0.3
As	0.91 ± 0.05	1.20 ± 0.14	1.24 ± 0.07	<0.2	0.260 (0.085–0.500)[§]	1.52 ± 0.10	<0.07	0.038 ± 0.007
Br	2.35 ± 0.11	33.3 ± 0.9	3.5 ± 0.2	0.69 ± 0.06	(0.65–53.3)[¶]	14.9 ± 0.5	1.57 ± 0.02	1.8
Ag	59.6 ± 1.5	65 ± 2	32.3 ± 0.9	23.6 ± 0.9	0.255 ± 0.196[‖]	2.3 ± 0.2	<0.05	–
Sb	0.72 ± 0.05	1.36 ± 0.18	1.84 ± 0.08	6.0 ± 0.2	(0.09–3)[¶]	0.96 ± 0.06	0.008 ± 0.003	0.013
Au	3.53 ± 0.07	0.94 ± 0.03	5.16 ± 0.10	2.92 ± 0.11	(0.012–0.66)**	3.92 ± 0.17	0.004 ± 0.003	0.001
Hg	10.5 ± 0.4	9.1 ± 0.8	11.4 ± 0.4	5.04 ± 0.18[††]	3.25 (0.5–12.2)[§]	2.13 ± 0.07[††]	0.040 ± 0.002 ($n = 6$)[††]	0.044 ± 0.004

*Certified values are equipped with uncertainty; values without uncertainty are non-certified (NIST 1993).

[†]Value ± combined uncertainty (coverage factor $k = 1$).

[‡]Unless otherwise stated.

[§]Iyengar and Woittiez (1988).

[¶]Iyengar *et al.* (1978).

[‖]Mean ± SD for 69 subjects not wearing Ag jewellery (Chojnacka *et al.* 2011).

**Range of arithmetic means for healthy populations from several countries not frequently wearing Au jewellery (Tadros and El Sweify 2011).

[††]RNAA.

NA, not analysed.

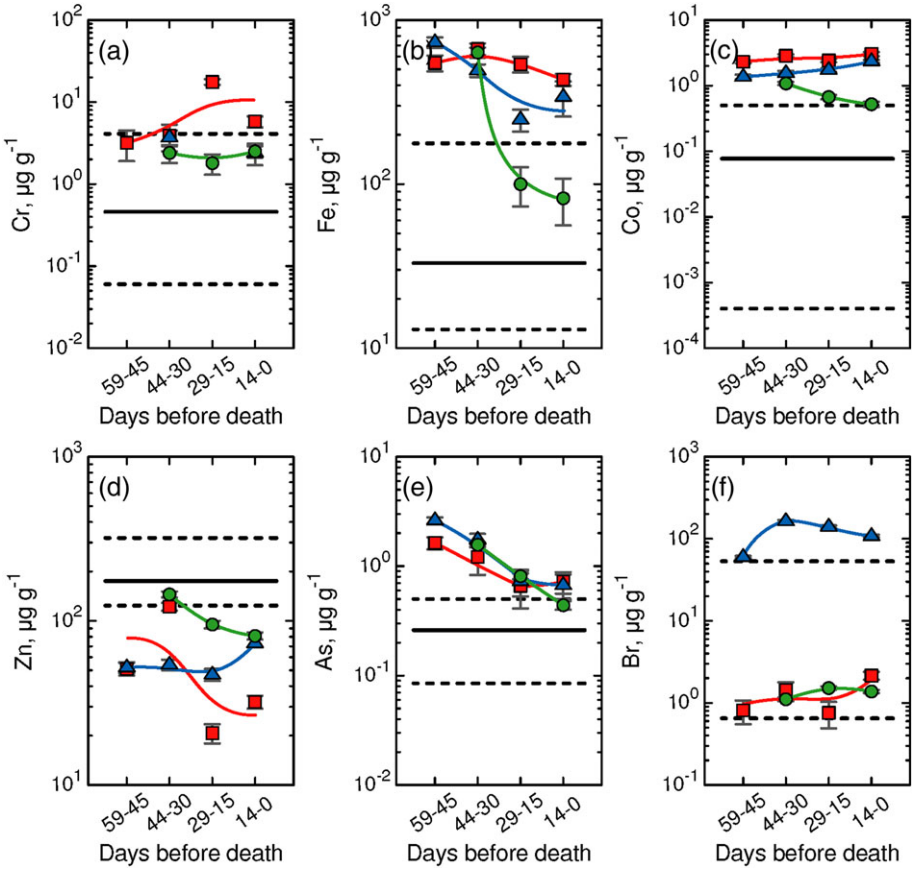

Figure 1 *Time trends of content of the elements (a) Cr, (b) Fe, (c) Co, (d) Zn, (e) As and (f) Br in hair samples TB38 (squares), TB39 (circles) and TB77 (triangles). The median and the range of 'normal' contents for the contemporary population (Iyengar and Woittiez 1988) are shown by full and dashed lines, respectively, except for Br, where only the range (dashed lines) is available (Iyengar et al. 1978). Missing experimental data for Cr are values below limit of detection. [Colour figure can be viewed at wileyonlinelibrary.com]*

mentioned decreasing trends for some of the elements (Fe, As, Ag, Au and Hg) towards his death present a much more complex issue. A possible reason could be an exposure lasting until some months before his death, but not extending back for years, as this would otherwise have been recorded in his bones (presented later in this section). This finding could possibly be related to Brahe's medico-alchemical activities, namely those concerning the preparation of Paracelsian medicines containing inorganic constituents. Of these, *Elixir Tychonis* (Figala 1972) is the most famous, and it is possible that Tycho Brahe could have tested or self-administered some of these medicines. If this was the case, the intake of the medicine, which must have taken place at, or some time before, 2 months prior to his death, was discontinued at that time. There is relatively little information about element contents in bodies of alchemists, the only case being analysis of Newton's hair (Spargo and Pounds 1979), performed in an attempt to explain his collapse in 1693. The composition of his hairs casts light on the exposure of active alchemists to different elements.

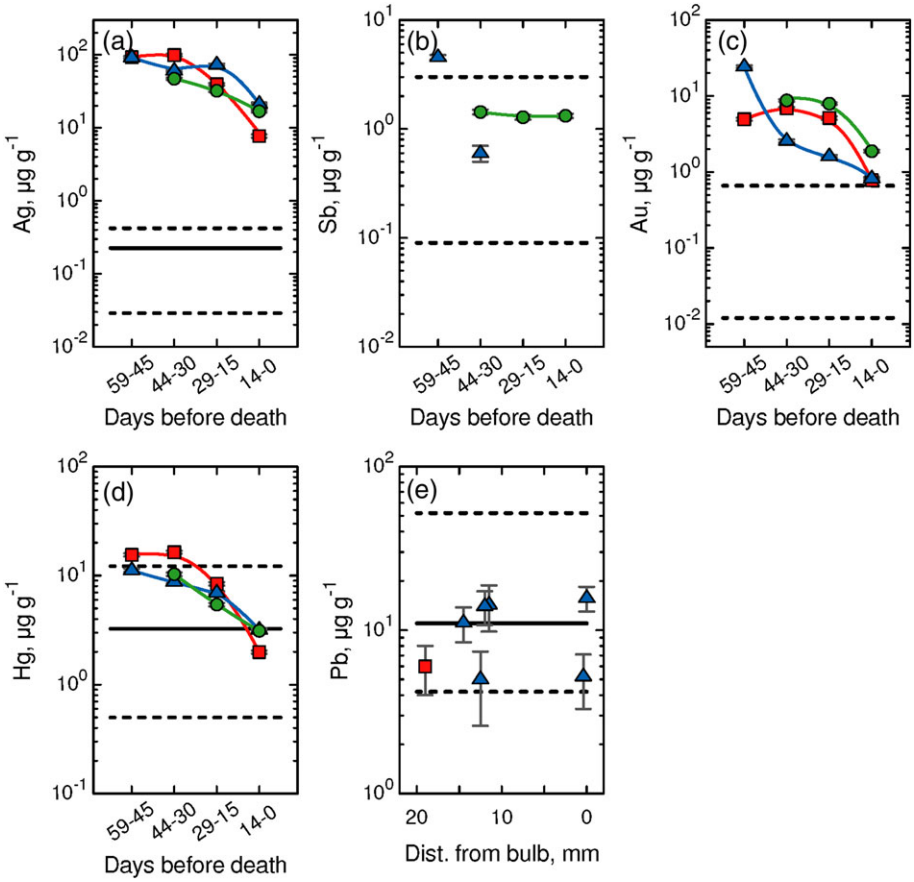

Figure 2 *Time trends of concentrations of the elements (a) Ag, (b) Sb, (c) Au, (d) Hg and (e) Pb in hair samples TB38 (squares), TB39 (circles) and TB77 (triangles). The median and the range of 'normal' contents for the contemporary population (Iyengar and Woittiez 1988) are shown by full and dashed lines, respectively, for the elements Hg and Pb. For Sb, only the range (dashed lines) is available (Iyengar et al. 1978), while for Ag full and dashed lines show the arithmetic mean ± SD (Chojnacka et al. 2011), respectively, and for Au only the range (dashed lines) is available (Tadros and El Sweify 2011). Missing experimental data for Sb are values below the limit of detection. [Colour figure can be viewed at wileyonlinelibrary.com]*

Bone samples

The element concentrations in the bone samples provide information about Brahe's long-term exposure to these elements, although exactly for how long a time depends on the turnover time of the specific bone tissue. The trabecular tissue has a turnover time of ~7% y^{-1}, while the cortical tissue has an approximate turnover time of ~2% y^{-1} (Reference Man 1975; Parfitt 1976, 2002).

The results for the analyses of the bone samples are given in Table 3, which also provides a comparison with concentrations reported for modern man and renaissance and medieval populations. Quite wide ranges of concentrations are reported in the literature for the contemporary population (Reference Man 1975; Iyengar and Tandon 1999). For the medieval and renaissance population in Northern Europe, there are also variations but they are centred around somewhat different mean values (Rasmussen *et al.* 2008, 2013b, 2015; Skytte and Rasmussen 2013), and in a Southern European population as well (Torino *et al.* 2015). One potential difference between

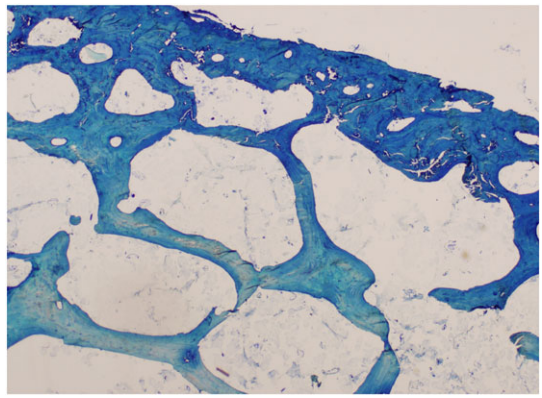

Figure 3 *A histological section from the sample obtained from iliac bone had normal architecture in both main components; that is, in cortical and cancellous bone. Cortical thinning was not proved and connectivity of bone trabecullae was preserved. Magnification 24×, stained using toluidin blue. [Colour figure can be viewed at wileyonlinelibrary.com]*

the concentrations reported for modern individuals (Reference Man 1975; Iyengar and Tandon 1999) and those for the renaissance and medieval population (e.g., Rasmussen *et al.* 2008, 2013b, 2015; Skytte and Rasmussen 2013; Torino *et al.* 2015) is that the former are primarily based on analyses of individuals who have not been interred in the ground, whereas the latter are based on buried individuals, whose remains have been in intimate contact with soil and humidity for several hundred years. The individuals interred in the ground can have been exposed to various processes, including decomposition and collapse of soft tissue on to the bone surface, which is particularly significant for the trabecular tissue, and diagenetic processes such as the invasion of small soil particles into the cracks in the bones or adhering to the bone lamellae in the trabecular tissue. Finally, Fe and Mn can have precipitated from the percolating groundwater. The bone samples of Tycho Brahe have indeed been buried in the ground for 300 years (until 1901, when his remains were transferred to a Sn coffin) and the soft tissues have decayed. Therefore, the results of the present work are expected to be more comparable to those of other interred renaissance and medieval individuals than to the results for modern non-interred individuals, although these can be used as guidelines when nothing else is available.

Mercury analyses were reported earlier (Rasmussen *et al.* 2013c), but the Hg concentration for the trabecular sample TB55 reported in the present work is different from that in Rasmussen *et al.* (2013c), because it is based on an analysis of a new sample, which has been mechanically decontaminated for dark particles as described above. The resulting Hg concentration is $14.6 \pm 2.4\,\mathrm{ng\,g^{-1}}$, which is considerably lower than the $92 \pm 1\,\mathrm{ng\,g^{-1}}$ reported in Rasmussen *et al.* (2013c). However, both values are very low; Rasmussen *et al.* (2015) deduced a threshold value of $300\,\mathrm{ng\,g^{-1}}$ for non-exposed medieval/renaissance populations for trabecular tissue samples. The collective results for Hg show that Tycho Brahe was not exposed to Hg beyond the level of the non-exposed part of the population. This excludes significant exposure to Hg in any connection; for example, gilding of instruments, or fabrication or intake of Hg-containing medicine, as has been observed in many medieval and Renaissance individuals (Charlier 2006; Rasmussen *et al.* 2008, 2012, 2013a, 2013b, 2013c, 2015; Fornaciari *et al.* 2011; Schwarz *et al.* 2013), including Sir Isaac Newton (Keynes 1995).

Arsenic concentrations in the bone samples are higher than the values for modern individuals (Reference Man 1975; Iyengar and Tandon 1999), but very similar to the values for

Table 3 *Element contents in bones in μg g^{-1} (unless otherwise given) determined by the indicated analytical methods, including quality control results for NIST SRM 1486 Bone Meal. Literature values for modern man and for renaissance and medieval populations are given in the last two columns.*

	TB55 Trabecular ICP-MS	*TB56A Cortical INAA*	*TB56B Trabecular INAA*	*TB57a Cortical ICP-MS*	*TB58 Cortical INAA*	*NIST SRM 1486 (N=6) ICP-MS*	*NIST value*	*Modern population**	*Renaissance and Medieval population*
	$x_i \pm u_i$[†]	$x_i \pm u_i$[†]	$x_i \pm u_i$[†]	$x_i \pm u_i$[†]	$x_i \pm u_i$[†]	$x_i \pm u_i$[†]	$x_i \pm u_i$[†]	*Range*	*Range*
Mg	1239±62	1190±170	930±130	1470±140	4770±230		4660±170	100 – 3900[‡]	650 – 2180[§]
Al	4.71±0.08	NA	NA	17.6±0.4	NA			1-5[¶]	5 – 450[§]
Ca,%	24.0±0.5	20±3	24±1	22±3	26.8±1.1		26.58±0.24	8.62 – 28.9[‡]	22.1 - 34.6[§]
Cr	< 2.96	1.0±0.3	< 0.4	< 2.96	< 0.4			2.75 – 10.8[‡]	
Mn	446±4	730±60	800±60	18.8±1.1	95±7		(1)	0.14 – 7.6[‡]	19 – 434[§]
Fe	174±20	571±16	488±11	849±17	106±4	97±9	99±8	31.2 – 532.5[‡]	31 – 17000[§]
Co	< 0.76	0.446±0.016	0.523±0.013	NA	0.065±0.005			0.0153 – 0.13[‡]	
Cu	< 3.04	< 25	< 20	< 3.04	< 15		(0.8)	0.19 – 22.6[‡]	0.6 – 113[§]
Zn	120±7	162±3	550±11	135±1.8	149±3	135±12	147±16	91 - 265.8[‡]	75 – 857[§]
As	< 0.92	< 0.5	0.39±0.07	< 0.92	0.46±0.07		(0.0006)	< 0.0024 – 0.012[‖]	0.9 - 6.5** 0.5 - 4.3[††]
Sr	186±1.7	168±11	187±7	137±2.6	NA	260±8	264±7	48.1 – 418	152 – 480[§]
Ag	< 0.24	< 0.2	< 0.1	< 0.24	< 0.1			0.022 – 0.039[‡]	
Sb	< 0.82	0.174±0.019	0.177±0.008	< 0.82	0.065±0.006			0.01 – 0.3[‡]	
Ba	7.27±0.10	<20	NA	19.0±0.3	NA			2.7 – 5.93[‡]	19 – 1090[§]
Au	< 0.3	0.021±0.007	0.038±0.002	< 0.3	0.008±0.002			0.00003–0.00039[‖] 0.001[¶]	
Hg[§,‡‡g]	0.0146±0.0024	0.036±0.001[‡‡]	0.030±0.001[‡‡]	0.036±0.013[§§]	NA			0.018 – 0.62[‡]	Cort. < 0.08[¶¶] Trab. < 0.30[¶¶]
Pb	111±1.1	NA	NA	72.7±1.4	NA	1.75±0.19	1.335±0.014	0.57 – 70.66[‡]	1.8 - 35.7[§] Up to 1835[¶¶]

*All values are given on dry mass basis. Results reported on fresh or dry mass basis were recalculated to dry mass using factors given in Iyengar *et al.* (1978)
[†]Value ± combined uncertainty (coverage factor k=1)
[‡]Iyengar and Tandon 1999
[§]Skytte and Rasmussen (2013)
[¶]Reference Man (1975)
[‖]Pietra *et al.* 1993
**Unpublished results from the CHART group
[††]Non-contaminated Mesolithic and medieval individuals Rasmussen *et al.* (2009)
[‡‡]RNAA
[§§]CV-AAS (Rasmussen *et al.* 2013c)
[¶¶]Rasmussen *et al.* (2015)
NA - not analysed

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archaeologically derived skeletons from the mesolithic, medieval and renaissance periods (Rasmussen *et al.* 2009; and unpublished results for more than 200 individuals). Tycho Brahe therefore seems not to have been exposed to more As during the last *c*. 5–10 years of his life than the contemporary renaissance population.

Magnesium, Cr, Zn, Sr, Sb and Ba all occur in concentrations in accordance with those seen in modern and renaissance individuals, and are not conspicuous in any way. Cobalt is slightly enriched compared to modern individuals. However, there are no data for Co on other thoroughly decontaminated renaissance individuals with which to compare. It is therefore our opinion that the determined Co concentrations are not conspicuously elevated. The same applies for Ag and Cu, where all the values were below their respective LOQs.

The Pb concentrations are $111 \pm 12\,\mu g\,g^{-1}$ for the trabecular sample TB55 and $72.7 \pm 8.0\,\mu g\,g^{-1}$ for the cortical sample TB57a. Lead has been suspected to accumulate in ancient human bones (Kosugi *et al.* 1986; Ericson *et al.* 1991). Rasmussen *et al.* (2015) reported Pb concentrations for both cortical and trabecular femoral bone tissue in 207 medieval and renaissance individuals from southern Denmark and northern Germany. They found that the unexposed populations in four rural cemeteries had Pb concentrations of less than $5\,\mu g\,g^{-1}$ in the cortical tissue and less than $7\,\mu g\,g^{-1}$ in the trabecular, whereas the populations in two urban communities exhibited much higher Pb concentrations, ranging from a few $\mu g\ g^{-1}$ to $1835\,\mu g\,g^{-1}$. Tycho Brahe lived in urban, rich and advanced societies, amongst the highest social classes, during his entire life—in Denmark, around Europe and in Prague. The Pb concentrations found in both tissues are in fine accordance with other similar individuals, and there is no reason to suspect that he had been subjected to excess Pb exposure beyond what was normal for his time and social class.

Aluminium, Fe and Mn normally occur in very low concentrations in the skeletons of modern individuals (Reference Man 1975; Iyengar and Tandon 1999), but are often much more abundant in archaeologically derived skeletons, where soaring values are occasionally encountered. This is mainly due to diagenesis (Hedges 2002; Skytte and Rasmussen 2013). Excess concentrations of Al can be caused by diagenesis in the form of invading soil particles, particularly clay particles, whereas excess concentrations of Fe and Mn can be caused by precipitation from groundwater (Keeley *et al.* 1977; Lopez-Gonzalez *et al.* 2006; Kuczomow *et al.* 2010; Rasmussen *et al.* 2013b, Skytte and Rasmussen 2013). The analyses of the bone samples of Tycho Brahe show that Al is hardly elevated at all, and the possibility that soil particles penetrated into the bone samples to any significant degree can therefore be excluded. Iron and manganese are somewhat elevated in most of the samples analysed. This indicates that the remains of Tycho Brahe could have been slightly exposed to percolating groundwater followed by precipitation of Fe and Mn onto the bones during the time he has been interred in the ground.

Gold, however, is the most conspicuous element. The values found in the three samples analysed by INAA range from 8 to $38\,ng\,g^{-1}$, thus exceeding the upper end of the normal range of Au for modern individuals by a factor of 21 to 97. We have found no data for renaissance individuals, but Au is an unlikely contaminant from soil, groundwater or decayed soft tissue. It is therefore likely that Tycho Brahe was exposed to excessive levels of Au in the last 5–10 years of his life.

There can be many routes of exposure to Au; for example, gold cutlery, gold-plated dishes or the addition of gold leaf to wine. Gold was ubiquitous throughout the higher social circles of Renaissance Europe. It is, of course, also possible that the excess Au originated from medication, possibly in a colloidal form. In any event, the decreasing Au concentration in the hair shows that the exposure to Au recorded in the bones was discontinued at least some months prior to the Brahe's death.

We presume that *post mortem* contamination only played a minor role for the distribution of the elements besides possibly Fe and Mn. The burial site is indoors, in a crypt under the floor of the Church of Our Lady before Týn. The photographic documentation from Heinrich Matiegka's opening of the grave in 1901 shows a corpse wrapped in rather well-preserved textiles, and with well-preserved skin and hair in several places. The surfaces of the bone samples were mechanically removed with a Dremel drill, making certain that only pristine bone material was used for analysis. The surface cleaning of the hair samples was performed using a standardized procedure supposedly capable of removing external contamination without significant influence on the endogenous element contents.

Histological examination

Nothing is generally known about Brahe's health status during the last years of his life, as well as shortly before his death, with the exception of the final 11 days (Janovský 2010; Rasmussen *et al.* 2013c). Histological examination of the bones was carried out, because histomorphometric analysis of bone specimens plays a role in the diagnosis and detection of treatment of metabolic bone diseases. The sample obtained from iliac bone had normal histological architecture in both main components; that is, in cortical and trabecular bone. When studying cortical and trabecular bone in polarized light, a clear lamellar pattern was visible, which is characteristic of normal bone. No woven bone structures were observed, which would have been indicative of some metabolic bone disorders. Connectivity of bone trabeculae was preserved. Minimal signs of osteoclastic resorption process were observed in both cortical and trabecular bone. We were not able to identify deep resorption cavities, which occurs in renal bone disease. Porosity of the cortical bone was not increased and cortical thinning was not proved.

Bone biopsy samples reflect both past and current bone cellular events in living people. Some parameters also remain preserved after death; for instance, the bone mass, which depends on the balance between resorption and formation of bone. In Brahe's case, the mineralization of the bone was preserved with quite normal parameters (Fig. 2). Very thin osteoid seams covering the surface of bone trabeculae were rarely observed. The trabecular volume was 19.8%, the osteoid relative surface was 0.7%, the trabecular diameter was measured to 161 ± 51 μm and the eroded surface area was 2.1%.

Histochemical staining reactions for Al were negative. The histochemical reaction for Fe was very weakly and diffusely positive in bone trabeculae.

A proper analysis consists of an accurate measurement of the dynamic and the static parameters, which are related to bone metabolism, which again is influenced by different organ functions. The data derived from a *post mortem* sample allow us to assess only some of the static variables; for example, bone volume, osteoid volume and trabecular diameter. The measurement of osteoblast and osteoclast activity was not possible, because *post mortem* autolytic processes had destroyed all the cells and non-mineralized tissues. Age- and sex-dependent variations both in static and dynamic parameters are well known (Rehman *et al.* 1994): the bone volume decreases significantly with age. At CUP, we obtained very similar results from a histomorphometric study of samples from a control group of Czech men (Povýšil, unpublished data). In the group of men over 60 years of age, the bone trabecular volume was 19.2 ± 5.0%, the osteoid relative surface 2.4 ± 1.1%, the trabecular diameter 139 ± 12 μm and the eroded surface 3.5 ± 1.5%. These values are very similar to our observations of the bone samples of Tycho Brahe.

Staining methods are used to enable separation of osteoid from mineralized bone in histological sections. The observations on the bone sample of Tycho Brahe show that only rare and only very thin osteoid seams composed of unmineralized bone matrix were identified. This means that process of bone mineralization was within the normal limits. This finding excludes the presence of disorders such as renal osteodystrophy, nutritional osteomalacia, vitamin D and calcium deficiency from gastrointestinal malabsorption syndromes, intoxication with certain metals and phosphate deficiency. Hypophosphatemic syndromes occur in acquired and congenital forms predominantly as a result of renal loss of phosphate secondary to a mesenchymal tumour, or in an altered renal tubular function as a consequence of a primary inborn error of phosphate transport in the proximal nephron.

Osteoporosis is a metabolic bone disease in which the amount of normally mineralized bone has been reduced to a level at which the risk of fracture occurring in the absence of trauma or in response to minimal trauma is increased. Osteoporosis without fracture, called 'osteopenia', can also occur. In the bone samples of Tycho Brahe, the value of the bone trabecular volume was normal, as well as the trabecular diameter. On the basis of these parameters, we can exclude osteopenia as well as osteoporosis.

Our results of the histological and histomorphometric examination of the bone sample from Brahe's iliac bone do not reveal any severe bone metabolic disorder. We can exclude osteomalatic disorder, which occurs as a complication of different renal, endocrine and gastrointestinal diseases, as well as osteoporosis or deposition of metals, specifically deposition of Al and Fe. The results of basic histomorphometric examination of the bone samples of Tycho Brahe were in good agreement with control data from a British study (Rehman *et al.* 1994) and with data from a control group of Czech patients examined at CUP.

CONCLUSIONS

Normal concentrations compatible with modern non-exposed individuals were found in the hair samples of Tycho Brahe for the elements Cr, Zn, Br, Sb, Pb and Hg. Anomalously high concentrations were found in the hair of Tycho Brahe for the elements Fe, Co, As, Ag and Au, which suggests that he had been exposed to these elements in the last 2 months prior to his death.

Decreasing time trends were observed for Fe, As, Ag, Au and Hg, which indicates that the exposure to these elements decreased during the 2 months before his death. There could be several possible sources of these elements; for example, alchemical activities or medicines, which he could have either manufactured or self-administered. On the other hand, the concentrations of these elements were relatively low, which makes it unlikely that the exposure could have caused any acute health problems, as was the case for Hg and Pb in Sir Isaac Newton (Keynes 1995).

The analysis of the bone samples revealed concentrations that were within the expected ranges for the following elements, all expected to be indigenous in the bone matrix: Mg, Ca, Cr, Co, Zn, As, Sr, Ag, Sb, Ba, Hg and Pb. Elevated concentrations above the background threshold values were found for Fe and Mn, which probably originate from contamination/diagenesis caused by deposition from percolating groundwater.

However, Au seems to be present in the bones of Brahe in concentrations exceeding those found in modern individuals. It therefore seems that Tycho Brahe was exposed to what must today be considered abnormal levels of Au in the last 5–10 years of his life. Whether this was also abnormal in the higher social circles in the renaissance cannot be ascertained. There are many possible sources for Au—for example, gold cutlery, gold plating, gold leaf added to wine and alchemy, but it is impossible to pinpoint a single source. However, the absence of other elements

which were usually used in alchemic experiments, such as As, Ag, Sb, Hg and Pb, makes it less likely that the source of Au was alchemy.

The histological and histomorphometric examination of the bones of Tycho Brahe revealed no severe bone metabolic disorders.

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